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MITIGATING ANTHROPOGENIC NITROGEN LOADING WITH CONSTRUCTED WETLANDS IN A BOREAL CLIMATE

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ACADEMIC DISSERTATION

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To my amazing children Konsta and Silja,

– I love you to The Moon and back.

ABSTRACT

Massive amounts of industrially-fixed reactive nitrogen (N) results in excess N amounts in the environment. High N loading from agricultural catchments and wastewater treatment plants can be mitigated by natural N removal processes in aquatic environments. These microbe meditated processes, mainly denitrification, remove the reactive N returning it back to the atmosphere mainly in the form of harmless N_2 gas. These processes occur in anoxic sediments and are dependent on the surrounding temperature. Generally, microbial N removal has been found to be efficient in wetlands, compared with other aquatic environments. In a cold climate, the process rates of microbial N removal are assumed to be low, however they are poorly understood. In this study, N removal rates were measured in a shallow agricultural wetland and in a wetland polishing treated wastewater situated in southern Finland. In the agricultural wetland, the influence of environmental factors were studied by conducting the study in field conditions. Seasonal denitrification rates were governed mainly by sediment temperature, followed by sediment oxygen conditions and water turbidity. Diurnally, denitrification was regulated by light affecting sediment oxygen conditions. Rates measured in the field merely during daytime may lead to underestimations of N removal potential of shallow wetlands. Nutrient retention and the contribution of N removal processes to the total N retention was estimated in the wetland receiving treated wastewater. The efficient N retention found was estimated to be mostly based on microbial processes, mainly on denitrification and slightly on anammox. In addition, the interference arising from organic carbon to nitrate measurements with optical sensors was studied and the information was used in improving the accuracy of sensor nitrate results in an agricultural brook. Increasing organic carbon concentrations was found to increase the nitrate concentrations by sensors and the effect was manifold in bog water. The interference was resolved by taking account the dissolved organic carbon in the correction of the sensor results. This study increases the information of actual N removing process rates and improves the knowledge of the extent constructed wetlands can remove excess N in cold climate, and provides information for the use of optical sensors in monitoring N loading.

TIIVISTELMÄ

Teollisen typensidonnan myötä ympäristöömme päätyy suuret määrät ylimääräistä typpeä. Vesistöjen korkeaa typpikuormitusta maatalousvalaisilta valuma-alueilta sekä jätevedenpuhdistamoilta voidaan vähentää hyödyntämällä vesiympäristössä tapahtuvia luonnollisia typenpoistoprosesseja. Nämä mikrobiologiset prosessit, tärkeimpänä denitrifikaatio, poistavat reaktiivista typpeä palauttaen sitä takaisin ilmakehään pääosin harmittomana typpikaasuna N_2. Kyseiset prosessit tapahtuvat hapettomissa sedimenttikerroksissa ja ne ovat lämpötilasta riippuvaisia. Kosteikoissa typenpoiston on todettu yleensä olevan tehokasta verrattuna muihin akvaattisiin ympäristöihin. Kylmässä ilmastossa näiden typenpoistoprosessien nopeuksien oletetaan olevan alhaisia, mutta niistä tiedetään hyvin vähän. Tässä tutkimuksessa typenpoiston nopeutta mitattiin eteläisessä Suomessa matalassa maatalouskosteikossa sekä kosteikossa, johon johdettiin puhdistettuja asumajätevesiä. Maatalouskosteikossa tehdyissä kenttäkokeissa tutkittiin ympäristötekijöiden vaikutusta typenpoistoon. Vuoden ympäri tehdyissä mittauksissa denitrifikaationopeutta säätelivät eniten sedimentin lämpötila, happiolosuhteet ja veden sameus. Vuorokauden ympäri tehdyissä kokeissa todettiin valon ja happiolosuhteiden vaikuttavan eniten denitrifikaatioon. Pelkästään päiväsaikaan tehdyt kenttämittaukset denitrifikaationopeuksista saattavat johtaa matalien kosteikkojen typenpoistopotentiaalin aliarviointiin. Puhdistamokosteikossa tutkittiin ravinneretention lisäksi typenpoiston osuutta mitatusta typenpidättymisestä. Typen pidättyminen kosteikossa oli tehokasta ja sen arvioitiin perustuvan suurimmaksi osaksi mikrobiologiseen typenpoistoon, pääosin denitrifikaatioon, mutta hieman myös anammoxiin. Lisäksi tutkittiin orgaanisen hiilen vaikutusta optisilla sensoreilla mitattuihin nitraattipitoisuuksiin. Kasvava orgaanisen hiilen määrä nosti sensoreilla mitattuja nitraattipitoisuuksia ja vaikutus suopitoisissa vesissä oli moninkertainen. Orgaanisen hiilen aiheuttama vaikutus maatalousojassa mitattuihin nitraattituloksiin korjattiin huomioimalla sensoritulosten laskennassa orgaanisen hiilen määrä. Tämä tutkimus tuo uutta tietoa rakennettujen kosteikkojen typenpoistoprosessien nopeuksista, rakennettujen kosteikkojen hyödyntämismahdollisuuksista kylmässä ilmastossa, sekä optisten jatkuvatoimisten nitraattisensorien käytöstä typpikuormituksen seurannassa.

LIST OF ORIGINAL PUBLICATIONS AND THE AUTHORS CONTRIBUTION

This thesis is based on the following publications:

I: Uusheimo S., Tulonen T., Aalto S.L., Arvola L. 2018. Mitigating agricultural nitrogen load with constructed ponds in northern latitudes: A field study on sedimental denitrification rates. Agriculture, Ecosystems and Environment 261, 71-79.

II: Uusheimo S., Huotari J., Tulonen T., Aalto S.L., Rissanen A.J., Arvola L. 2018. High nitrogen removal in a constructed wetland receiving treated wastewater in a cold climate. Environmental Technology and Science 52, 13343-13350.

III: Uusheimo S., Tulonen T., Arvola L., Arola H., Linjama J., Huttula T. 2017. Organic carbon causes interference with nitrate and nitrite measurements by UV/Vis spectrometers: the importance of local calibration. Environmental Monitoring and Assessment, 189:357.

The publications are referred in the text by their Roman numerals (**I-III**).

I: Uusheimo had the main responsibility for writing the article, designing the study and for data collection with the participation of TT and LA. Data analysis was done together with SA.

II: Uusheimo had the main responsibility for writing the article, data collection and analysis. Study was designed jointly with all co-authors.

III: Uusheimo took part in the data collection concerning experiment III. Data analysis was done jointly with the group. Uusheimo had the main responsibility for writing the article.

CONTENTS

ABBREVIATIONS

ANAMMOX	anaerobic ammonium oxidation
BNF	biological nitrogen fixation
C	carbon
CW	constructed wetland
D_n	denitrification coupled to nitrification
D_w	denitrification based on water column nitrate
D_{14}	denitrification of ambient nitrate
D_{15}	denitrification of labeled nitrate
DIN	dissolved inorganic nitrogen
DNRA	dissimilatory nitrate reduction to ammonium
DO	dissolved oxygen
DOC	dissolved organic carbon
DON	dissolved organic nitrogen
IPT	isotope pairing technique
MLR	multiple linear regression
N	nitrogen
N_r	reactive nitrogen
N_2	nitrogen gas
N_2O	nitrous oxide
NH_3	ammonia
NH_4-N	ammonium nitrogen
NO	nitric oxide
NO_x-N	nitrate + nitrite nitrogen
NO_2-N	nitrite nitrogen
NO_3-N	nitrate nitrogen
O_2	oxygen
OC	organic carbon
ON	organic nitrogen
OPD	oxygen penetration depth
P	phosphorus
POC	particulate organic carbon
PON	particulate organic nitrogen
PO_4-P	phosphate phosphorus
SLR	simple linear regression
TN	total nitrogen
TP	total phosphorus
TOC	total organic carbon
WWTP	wastewater treatment plant

Äiti, – mitä sä oikein teet työksesi?

- Mä teen väitöskirjaa.

Hei mäkin teen värityskirjaa!!

-Silja 4v.-

1 INTRODUCTION

1.1 THE INVENTION THAT CHANGED THE WORLD FOREVER

The amount of nitrogen (N) on earth and the atmosphere is higher than the amount of all other major elements (carbon, oxygen, phosphorus, sulphur) combined. Since nearly 80% of air is consisted of di-nitrogen gas (N_2) which is not available to most of the organisms, N is the least available element to life. The two N atoms of N_2 gas molecule are connected by one of the strongest bonds found in nature, which only some microbes and lightning are able to brake. This microbiological process, on which the availability of N on earth before human interference was based, is known as biological N-fixation (BNF). All other forms other than N_2, whether reduced, oxidized or organic, are called reactive N (N_r).

According to many scientists, perhaps the greatest invention modifying the history of earth and mankind might have taken place in 1908 when Fritz Haber discovered how N_2 could be converted into N_r as in ammonia (NH_3). This invention, awarded by the 1918 Nobel Prize in chemistry, enabled the massive human population growth allowing food production to the growing demand with the industrial-scale fertilizer production invented by Carl Bosch. Another side of the story was to satisfy the increasing needs for military purposes including explosives and chemical warfare. (Galloway et al. 2013; Sutton et al. 2011). Without "Haber-Bosch N", the current world population would be 3.5 billion, meaning half of the world population is depending on the food produced by industrial fertilizers (Erisman et al. 2008). In Europe, North America, Latin America, India and China, the food supply is growing faster than the population. This exceeding food supply, which started as the "green revolution", has turned into a "livestock revolution" where industrially fertilized grain and grass is fed to the livestock in confined conditions. As a result, developing countries are consuming increasing amounts of inexpensive meat and dairy (Sutton et al. 2013).

"It may be that this solution is not the final one. Nitrogen bacteria teach us that Nature, with her sophisticated forms of the chemistry of living matter, still understands and utilizes methods which we do not as yet know how to imitate. Let it suffice that in the meantime improved nitrogen fertilization of the soil brings new nutritive riches to mankind and that the chemical industry comes to the aid of the farmer who, in the good earth, changes stones into bread."

"Ammonia synthesis from its elements", Nobel lecture
-Fritz Haber

1.2 CONSEQUENSES OF EXCESS REACTIVE NITROGEN

The invention of Haber-Bosch N has more than doubled the global N-fixation (natural N-fixation 203 Tg N yr^{-1}) mainly by industrial fertilizer production, but also with agricultural practices utilizing BNF in agriculture and with combustion processes in transport, energy production and industry (210 Tg N yr^{-1}) (Fowler et al 2013). According to scientists the disadvantages are already exceeding the advantages by making N pollution one of the major environmental challenges of this century (e.g. Brink & van Grisven 2011; Sutton et al. 2011). The concept of "nitrogen cascade" is used to describe the phenomenon where one nitrogen atom is participated, reused and cycled biogeochemically through several different processes in terrestrial, aquatic and atmospheric environment. This causes many environmental problems like freshwater, marine and terrestrial eutrophication, climate change, poor air quality, tropospheric ozone formation and stratospheric ozone loss (Galloway et al. 2003).

Anthropogenic eutrophication, which is recognized as the most challenging problem in surface waters due to excess N and phosphorus (P) (Smith et al. 2006; Smith & Schindler 2009), is often first observed as rapid increase in primary production leading to decreased transparency of the water column. In long-term, many undesirable ecological changes are found in the aquatic system, like oxygen depletion and generally impaired water quality (Camargo & Alonso 2006; Smith & Schindler 2009). Since sulphur (S) emissions have been successfully reduced in the late 20th century, more constant emissions of N_r have become the main reason for acidification in many freshwater systems (Camargo & Alonso 2006). Both eutrophication and acidification contribute to biodiversity loss in the long run.

At global scale, air deposited N_r is estimated to be responsible of 5–15% of biodiversity loss (Erisman et al. 2013). The most remote or elevated areas away from direct human influence have been found to be exposed to N_r deposition (Hundey et al. 2016; Wolff 2013). N_r related air pollution causes severe negative health impacts on humans. Upper limit values are most typically exceeded in urban areas and areas near farm animal housing. Every year approximately 500 000 premature deaths have been estimated to be caused by N_r related air pollution in EU area (Erisman et al. 2013).

N_r is linked in many ways to climate change. Northern peatlands contain about 1/3 of the world´s organic C in soils (Gorham 1991). High levels of N_r deposition can amplify global warming by decreasing C storage in peatlands. The most important C sequestering group of peat forming mosses *Sphagnum,* is known to suffer from N_r deposition (e.g. Granath et al. 2012). As a result, C storage in soils (Mack et al. 2004) and growth of *Sphagnum* are decreased (Berendse et al. 2001). Nitrous oxide (N_2O) is a strong greenhouse gas with a

long lifetime of 120 years in the atmosphere. The concentration in the atmosphere had exceeded the pre-industrial times by 20% already in 2011 (IPCC 2013). From 2011, N_2O has been the third largest contributor to anthropogenic radiative forcing affecting climate change (Myhre et al. 2013). N_2O is also recognized to be the main substance responsible for the depletion of stratospheric ozone (O_3) which protects all living organisms from the biologically harmful ultraviolet light (Ravishankara et al. 2009).

Because N_r is highly mobile and escapes unintentially to the environment, N use-efficiency (NUE) especially in the meat-based food chain is poor (Bouwman et al. 2013). It should be noted that about 2/3 of the produced grain is fed to livestock instead of people (Poore & Nemeck 2018). The global average for the efficiency of converting vegetal protein into animal protein is only 12%. As a result, the amount of N_r used in the beginning of food production is 10-fold compared with the N_r in the food consumed by humans (Erisman et al. 2013). Furthermore, the dietary intake of proteins in the developed countries exceeds the recommendation (WHO 2007).

Most of the N_r abatement strategies regarding the food sector have been focusing on the production side rather than the consumers even though the motivation to what is produced arises from consumers preferences. N footprint is an indicator directed to consumers and is used to quantify the losses of N_r to the environment and the use of energy in food production (Shibata et al. 2017). Comparing the N footprints from different countries and regions, scientists have identified options to reduce N_r pollution, e.g. improving the NUE, reducing the wastage of food and choosing a diet with less animal protein. For example, in a study conducted in United Kingdom, Stevens et al. (2014) found that lowering protein intake of food to the recommended level by the World Health Organization (WHO) could allow the N footprint to be 33% smaller.

Rockström et al. (2009) were the first to define the planetary boundaries on earth including proposed quantifications for seven variables: climate change, ocean acidification, stratospheric ozone, N cycle, P cycle, freshwater use, land system change and biodiversity loss. Humans could operate safely by staying within the planetary boundaries, but crossing the quantified threshold might result in catastrophic changes threatening sustainable human life. They estimated that we have already transgressed three of these boundaries of which one is the biogeochemical cycle of N. They estimated that the amount of produced new N_r should be 25% of its current amount. Interventions to the N_r creation and management have been suggested (e.g. Galloway et al. 2008) and an International Nitrogen Management System (INMS) is underway for the development of policy for global N management (www.inms.international).

1.3 NITROGEN REGULATION, LOADING AND IMPORTANCE IN FINNISH WATERBODIES

Currently, international regulation for global management of N is still lacking. Regulation of N leakage and emissions in agricultural sector has not been as developed as in other areas like emissions in transport, wastewater or industry (Fowler et al. 2013). Within the globe, Europe is a hotspot in the use of anthropogenic N and the anthropogenic input of N is in average 5-fold the natural N_2-fixation rate (Billen 2011). In the European Union (EU), the Water Frame Directive (WFD, 2000/60/EC) aims to the good status for all the waterbodies through holistic and intergrated approach and has lead to the implementation on several directives concerning N loading to waterbodies. The Nitrates Directive (91/676/EEC) aims to reduce and prevent surface and groundwater from NO_3 pollution from agricultural sources. The Groundwater Directive (2006/118/EC) and the Drinking Water Directive (98/83/EC) set a concentration limit to 50 mg NO_3 l^{-1}. The Urban Wastewater Directive (91/271/EEC) sets rules for urban wastewater and certain industrial wastewater collection and treatment. Specifically, it obligates municipals to treat the wastewater of 2000 inhabitants at least biologically and as for cities over 10 000 inhabitants the treatment has to be more efficient, i.e. including enhanced N removal. In Finland, the Nitrates Directive is implemented by national Nitrates Decree (VNa 1250/2014) in the whole country because all freshwater bodies were originally classified as "sensitive", i.e. being vulnerable to eutrophication.

According to the latest report of European waters published by the European Environmental Agency (EEA 2018), only 38% of European surface waters were classified as good or high in ecological condition. Areas were generally found to be affected by diffuse nutrient and pesticide pollution mainly originating from agriculture. NO_3-levels decreased in European rivers from 1992 to 2015 by 20% in average, but 18% of the groundwater areas were affected by excess NO_3, the most common pollutant found in groundwaters. Compared with other EU countries, NO_3 levels of surface and groundwaters are low in Finland. However, the Environmental Implementation Review Country Report of Finland 2017 states two main challenges for Finland in executing EU environmental policy: reducing agricultural diffuse nutrient loading and improving air quality by reducing nitrite oxides (NO_2) in Helsinki area (EC 2017).

Comparing the two (2008–2011, 2012–2015) implementation periods of the Nitrates Directive in Finland (Mitikka 2017), a slightly increasing trends in surface water NO_3^- were found in 23 rivers, 13 coastal sites and 5 lakes (out of 178 sites). Between 2012–2015, the concentration limit of NO_3^- for surface water (25 mg l^{-1}) exceeded in 9 rivers, the highest concentration being slightly over 40 mg l^{-1}. One decreasing trend was found in River Porvoonjoki partly

due to the enhanced N removal at the wastewater treatment plant (WWTP) highlighting the regional importance of point sources. As for groundwaters, most of the NO_3^- concentrations were below 25 mg l^{-1} and the threshold (50 mg NO_3^- l^{-1}) was exceeded in 4 areas (out of 19 areas) affected by agriculture.

Between 2008 and 2016, Finnish rivers transported 82 000 tons of N on average per year to the Baltic Sea which equals 11% of the total N load (FEI 2018). Unlike with P, the trend of N loading is not declining. Approximately half of the Finnish N load originates from agriculture, 19% from air deposition and 17% from municipal wastewaters (FEI 2017). In general, riverine N fluxes are strongly associated with the volume of agriculture in the area. In Finland, the agricultural area takes only 7% of the entire land area (Natural Resources Insitute of Finland, June 15 2018), but the fields are generally situated near lakes, rivers or the sea coast. 87% of the cultivated area is boarded to the waterbody either directly (13%) or through a drainage channel (74%) (Puustinen et al. 1994). Agriculture is concentrated in western and southern parts of the country and in these areas agriculture is often responsible for most of the TN loading to waterbodies (Lepistö et al. 2006). Forestry is more intensive in eastern and northern parts of the country (Lepistö et al. 2006). Boreal forests are typically N-limited, so the inorganic N is efficiently retained in the system (Kortelainen et al. 2006). Generally, most of the N transported by boreal rivers and streams is in dissolved organic form (DON) (Lepistö et al. 2006; Kortelainen et al. 2006) which is important even though DIN is considered as the most significant N compound promoting algal growth in N-limited inland and coastal waters. DON can be utilized directly or undirectly by bacteria and phytoplankton (Berman & Bronk 2003; Seitzinger & Sanders 1997) and it is subject to photochemical degradation and modification. Total organic N (TON) export can be generally explained by peatland proportion in the catchment (Sarkkola 2012) and total organic C (TOC) and TON export are found to be strongly correlated (Lepistö et al. 2008). Climate change and increasing temperatures may lead to enhanced mineralization rates which has been associated with the increasing TOC export in boreal rivers, so the same effect may be seen as in increasing TON amount in waterways (Sarkkola 2012).

Eutrophication is one of the main environmental problems facing lakes, rivers and sea areas and has increased heavily since the 1960s with more than 400 marine sites around the world (Diaz & Rosenberg 2008). In the semi-enclosed, brackish and shallow Baltic Sea, N is generally the limiting nutrient in the Gulf of Finland, the Archipelago Sea and the Bothnian Sea (e.g. Granéli et al. 1990; Lehtoranta 2003). Primary production in freshwater systems is generally controlled by P (Schindler 1977; Wetzel 1983), but more recent studies have found that these systems can also be limited by N (Elser et al. 2007; Lewis et al. 2011; Paerl 2016). N limitation or N & P co-limitation are often related situations such as 1) shallow and well mixed waterbodies, 2) highly P-rich systems and systems with very low N load, 3) summertime N

limitation in an eutrophied waterbody (Durand et al. 2011). In a large meta-analysis, including 653 freshwater, 243 marine and 173 terrestrial experiments of growth responses to N and P additions, Elser et al. (2007) reported equally strong N and P limititation in freshwater systems. Pietiläinen & Räike (1999) studied the nutrient limitation in 174 lake and 32 river sites in Finland. Based on the mineral nutrient -ratios they concluded that 64% of lakes and 38% of rivers were limited by P, 13% of lakes and 31% of rivers were limited by N and the rest were limited by both. The primary production in large lakes were highly controlled by P, whereas in heavily eutrophied waterbodies N was often found limiting as a consequence of high internal loading in anoxic sediment conditions returning large amounts of P into water from the sediment.

Currently, P-reduction policy has dominated the water management in freshwaters, but the debate of reduction strategies goes on. Many scientists today support a more holistic view and encourage to the reduction of both nutrients (e.g. Finlay 2013; Durand 2011; James et al. 2005; Paerl 2009; Shatwell & Köhler 2018). It is argued that eutrophication problem should be evaluated in a larger scale, as waters run from lakes via river to the sea. Although controlling P restricts the growth of primary producers in the upstream waters, it also reduces the uptake of N into biomass. As a concequence, more N is discharged downstream and perhaps eventually to N-limited coastal areas instead of biomass mineralization in upstream lake sediments (Bernhardt 2013; Finlay 2013). The supporters of "P reduction only" have stated that the need for N in the ecosystem can be supplied by the N_2-fixers anyway (Schindler 2008; Higgins et al. 2018). However, this is critizised to be based on inadequate consideration of the environmental factors that inhibit N_2-fixation (like the presence of oxygen, excluding the heterocystous species) (Pearl 2009). It has been also argued that the conditions for optimal N_2-fixing are highly seasonal and temporary (Howart & Marino 2006) and that the deficiency of iron may restrict the activity of nitrogenase enzyme (Paerl et al. 2004). N_2-fixing cyanobacteria do have the advantage to exploit N from the atmosphere (e.g. Paerl & Otten 2013) and generally, cyanobacterial blooms occur typically in late summer under warm conditions. Over the summer, inorganic N concentration in the warming water decreases because of the enhanced microbiological N removal. Lower N/P -ratio favor the cyanobacteria capable of N_2-fixing and some toxic species do cause a risk to humans and animals. Cyanobacterial blooms are a common phenomena during summer in many eutrophied lakes as well as in the Baltic Sea where the worst cyanobacterial blooms for a decade were observed in 2018 (Fig. 1).

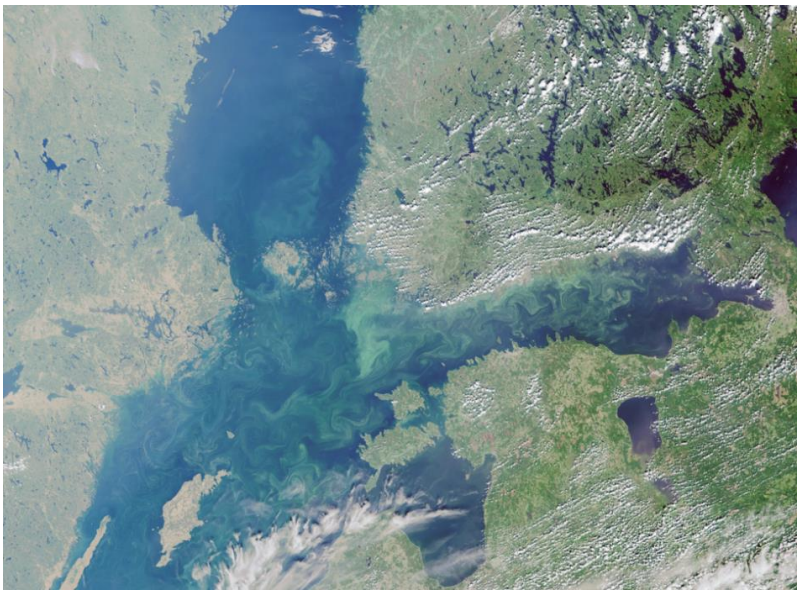

Figure 1. Spaceview of a cyanobacterial bloom in the Baltic Sea on July 16, 2018 (ESA Copernicus Sentinel Data, published by Finnish Environmental Institute SYKE).

Typically in an eutrophied waterbody, low oxygen concentrations i.e. hypoxia (<2 mg O_2 l^{-1}) or even total oxygen depletion (0 mg O_2 l^{-1}) are often detected in the bottom waters and sediments as a result of mineralization of high biomass produced in the upper water layers. Eventually, the oxygen depletion leads to P release from the sediment causing internal P loading (e.g. Funkey et al. 2014; Lehtoranta 2003) and switching N removal processes in the sediment towards recycling of N (Jäntti & Hietanen 2012). The persistency of oxygen depletion varies and may be interrupted along seasonal changes and mixing of water layers. Unlike freshwaters, the water column in the Baltic Sea is stratified also with different levels of salinity and the hypoxia may be persistent (e.g. Diaz & Rosenberg 2008). Since the 1950s, increased nutrient inputs have caused dramatic rise in hypoxic areas in the Baltic Sea (Carstensen et al. 2014). Severe O_2 conditions are the case especially in the area of Baltic Proper, where the deepest bottom water oxygen conditions recover only by oxygen-rich salt water pulses from the North Sea. During 2017, nearly 1/3 of the Baltic Proper was found hypoxic and 18% anoxic (Hansson et al. 2017). Continuously anoxic bottom sediment releases toxic hydrogen sulphide (H_2S) and the bottom sediment turns into a “dead zone” with the death of benthic animals.

Luckily, the entire N loading is not delivered to the Baltic Sea, but is being microbiologically returned to the atmosphere or storaged in sediments during its way through the waterbodies. N retention in the watershed depends on climatic factors, like temperature, runoff and water residence time (Lepistö et

al. 2006; Billen 2011). According to Lepistö et al. (2006), 22% (0-68%) of the N input in Finnish rivers was retained. The retention was highest (avg 48%) in the areas with a high lake percentage (> 10%) and lowest in coastal regions (avg 5%) with very few lakes (< 2%). Marine Strategy Framework Directive of European Commission (2008/56/EC) and Baltic Sea Action Plan by Helsinki Commission (HELCOM) aim to achieve environmentally good status in the Baltic Sea. In the latest status report of 2011–2015, the goals of nutrient thresholds were not achieve generally. Total N levels were the same compared with the levels between 2007–2011 and as for P, an increase was detected in three sub-basins, e.g. in the Gulf of Finland (HELCOM 2017). Rankinen et al. (2010) studied nutrient loading trends between 1985–2012 in 20 Finnish river basins. They found an increase in seasonal temperatures as well as in total N concentrations. With warming climate, precipitation has been predicted to increase in North Europe (Dore 2000; Falloon & Betts 2010). Increased N load can be expected to river-systems and eventually, promoting the eutrophication in the Baltic Sea since N export from watershed is correlated with precipitation (Arvola et al. 2015) and Baltic Sea N load is correlated with river runoff (Voss 2011). Coastal areas are generally considered to work like "a filter" removing nutrients efficiently. However, despite the increasing N loads, the natural N removal in the river estuaries is not expected to increase meaning limited reduction capacity of N (Hellemann et al. 2017; Silvennoinen 2008). Asmala et al. (2017) estimated that only 16% of the N was removed in the coastal area. Furthermore, Jäntti (2012) studied N cycling in the northern Baltic and concluded that the natural N removal was decreased compared with earlier rates because of increased hypoxia. Alltogether, these results highlight the importance of the N reduction activities conducted already in the catchments of inland areas.

1.4 AQUATIC NITROGEN CYCLE

N cycle in aquatic environments is complex and primarily meditated by microbes. Aquatic N is continuously transformed and recycled within the ecosystem, but also between aquatic and terrestrial ecosystems and the atmosphere. All forms of N_r are involved: dissolved inorganic DIN (NO_3^-, NO_2^-, NH_4^+) and dissolved gaseous forms (NH_3, N_2, N_2O, NO), dissolved organic N (DON) and particulate organic N forms (PON). DIN is directly available for primary producers and builds up in organic biomass in microbes, algae, plants and aquatic animals. Supply of N in all ecosystems was based almost entirely on biological N_2-fixation before humans perturbated the N cycle. In addition to N-fixation, NH_4^+ is produced in decomposition of organic matter, like dead plants and animals (ammonification). Aquatic DIN pool is not only used for nitrogenous nutrition for organisms, but some microbes also use it for energy metabolism in different N cycling processes: nitrification,

denitrification, anaerobic ammonium oxidation (anammox) and dissimilatory nitrate reduction to ammonium (DNRA) (Fig. 2). In the presence of oxygen (O_2), NH_4^+ is sequentially oxidized into NO_2^- and NO_3^- in a nitrification process (Fig 2.). It is not affecting the N budget of the system, but simply changing the oxidation state of the nitrogenous compound. Nitrification acts as an important link to denitrification which is the most important N removing process in freshwaters.

Denitrifiers are mostly heterotrophic microbes gaining their energy from the decomposition of organic matter, i.e. particulate organic carbon (POC) or dissolved organic carbon (DOC). Denitrifiers have the advantage to switch their metabolism from aerobic to anaerobic if O_2 becomes limiting. In that case, instead of O_2 they use NO_3^- as an electron acceptor in their respiration. NO_3^- is reduced by different reductase enzymes on each step and finally removed mainly as gaseous N_2 or N_2O from water to the atmosphere (Fig. 2). The capability to denitrify is widespread among bacteria, but all denitrifiers do not carry the gene encoding the last step, the nitrous oxide reductase (i.e. N_2O > N_2), and this has been found to be connected to N_2O accumulation in different boreal lakes (Saarenheimo et al. 2015). Furthermore, the reductase of the last step is very sensitive to the presence of oxygen and is inactivated switching the end product of denitrification being N_2O rather than N_2 (Ward 2015). However, generally the relative amount of N_2O compared with N_2 is low in natural aquatic systems (e.g. Silvennoinen 2008). Heterotrophic denitrification occurring in sediments is considered as the most significant N removal pathway in freshwater systems like wetlands, ponds, lakes and rivers (e.g. Mitch & Gosselink 2015; Seitzinger 1988) while autotrophic denitrification in which inorganic reduced compounds like H_2S are used as energy source, is more important in marine sediments (e.g. Shao et al. 2010). Denitrification is known to be promoted by higher temperature (e.g. Dawson & Murphy 1972) and also higher NO_3^- availability (e.g. Aalto et al. 2018; McCrackin & Elser 2010). In addition, the availability of labile OC may be limiting heterotrophic denitrifiers especially in high NO_3^- concentrations (e.g. Grebliunas 2015; Songliu 2009). Generally, large aromatic and partly degraded alloctonous OC is considered not as bioavailable as small and fresh autochtonous OC (Guillemette & del Giorgio 2012; Tulonen et al. 2000). DNRA is a process which competes for the same electron acceptor (NO_3^-) with denitrification. In DNRA, NO_3^- is reduced to NH_4^+ and N is recycled in the system. High C:N ratio is known to favor DNRA over denitrification, even though N_2 production can still be substantial if NO_3^- is available (Hardison et al. 2015; Kraft et al. 2014).

In addition to denitrification, anammox is another N_2 producing microbiological process which has been found to be important especially in marine environment (e.g. Dalsgaard et al. 2005; Jetten et al. 2009). In anammox, NH_4^+ is oxidized with NO_2^- producing harmless N_2 in anaerobic

conditions. Originally, anammox was found in WWTP in the 1990s and since then has been attracting a great deal of interest among wastewater treatment industry. As an autotrophic process, no external C source is needed meaning economic savings and anammox is currently applied in many full-scale WWTPs (Hu et al. 2013). Although anammox bacteria have been found in several natural and man-made environments (Ni & Zhang 2013; Oshiki et al. 2016), no anammox activity has been found in Finnish lakes (Holmroos 2014; Rissanen 2012). This may be explained with a low number of studies. In brackish environment like the Gulf of Finland, anammox has been estimated to contribute <20% to the total N removal (Hietanen 2007; Hietanen & Kuparinen 2008).

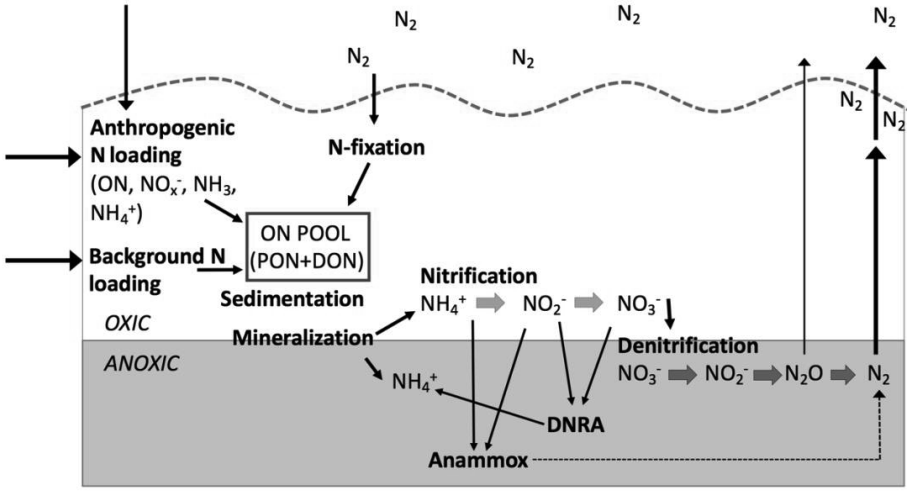

Figure 2. Simplified illustration of the aquatic nitrogen (N) cycle.

1.5 NITROGEN REMOVAL IN WETLANDS

In the western history, wetlands have been considered as wastelands; unproductive and a little scary places even among the studied. In 1732, Swedish botanist Carl Linnaeus traveled to the peatlands of Lapland and described his experience in posthumously published book (Smith 1811) like this:

> *"Shortly afterwards began the muskegs, which mostly stood under water; these we had to cross for miles; think with what misery, every step up to our knees. The whole of this land of the Lapps was mostly muskeg, hinc vocavi Styx. Never can the priest so describe hell, because it is no worse. Never have poets been able to picture Styx so foul, since that is no fouler".*

Gradually, the general perception of wetlands being hostile and useless environment is changing. Nowadays, wetlands are often called as "the kidneys of the landscape" or "the ecological supermarkets", because they purify polluted waters and support high biodiversity. Wetlands also provide many other important functions, "ecosystem services", like the stabilization of water supply, decreasing flood peaks, recharging groundwaters, supporting wildlife, storaging carbon, offering food, fiber and recreation possibilities (Millenium Ecosystem Assessment 2005; Mitsch et al. 2015). Unfortunately, population growth, urbanization and food production have resulted in massive loss of natural wetlands. The first global assessment of the wetland loss by Davidson (2014) reports that only about 13% of the natural wetlands remain since 1700 and this loss was the most rapid during 20th century when 70 % of the wetlands were destroyed. Between 1970 and 2008, 40% of the wetlands left were wiped out, most being small freshwater wetlands (RAMSAR 2015). Most European wetlands have been drained during the last 80 years and the remaining are located in the northern countries (Verhoeven 2014). In Finland, almost 2/3 of the original peatland area has been drained mainly for forestry and this has been especially intensive in south and east Finland where 90% have been drained (Turunen 2008). At the moment, the global loss of wetlands still continues and we are decreasing earth´s natural capacity to remove excess N_r from waterbodies.

There are number of definitions for a wetland, but perhaps the broadest definition comes from the Ramsar Convention on Wetlands (1971): "*Wetlands are areas of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water the depth of which at low tide does not exceed six metres*". Constructed wetlands (CWs) are built to mimick the purifying processes of natural wetlands, which are considered more efficient in N retention than lakes or rivers (Saunders & Kalff 2001). In addition, they are regarded as "hotspots" of denitrification (Mitsch et al. 2001; Seitzinger et al. 2006). When soil conditions change from oxic to anoxic in wetlands because of higher water saturation, N is processed differently producing mainly reduced N substances like N_2. In a review of 57 natural wetlands by Fisher & Ackremann (2004), 80% resulted in retaining N, 13% in N release and 7% showed not change in N load. Since the 1970s, CWs have been widely utilized all over the world to treat many kinds of wastewaters such as domestic sewage (often referred as "treatment wetlands"), agricultural and urban runoff, industrial wastewater, mine drainage and landfill leachate. Their design parameters (e.g. type, retention time, depth) depend on the type and the amount of wastewater to be treated (e.g. sewage, agricultural) relative to the environmental conditions (e.g. temperature, oxygen). Treatment wetlands are generally classified based on hydrology (free water surface flow FWS and subsurface flow SS) and vegetation (mainly emergent and submergent). The

reported efficiencies of CWs vary from 41–55% for TN, 39-84% for NO_x^--N and NH_4^+-N in treatment wetlands (Vymazal 2007) and in agricultural wetlands 4-85% (Vymazal 2017). Generally, CWs are considered as efficient, sustainable and a low-cost option to treat wastewaters (Lee et al. 2009; Mitsch & Gosselink 2015; Søvik et al. 2006; Wu et al. 2015). A large-scale study conducted in Minnesota river basin estimated that wetlands were five times more efficient in removing nitrate than agricultural practices, like land retirement or crop cover (Hansen et al. 2018). They stressed the importance of wetland positioning in the river network in reducing N load. This is sensible, as NO_x-N concentration together with residence time are among the most important factors in achieving efficient wetland N removal (e.g. Land et al. 2016).

N removal in northern CWs may be less efficient, because microbially mediated processes are temperature-dependent. In Finland, the average yearly temperature in south and central parts of the country is +1-5 ºC and in northern Finland between 0 and -2 ºC (FMI 2018). High variation in temperatures between seasons is typical with permanent snow cover during winter. Mean daily temperature in summer stays above 10 ºC, but in winter it is much lower (<0 ºC). Precipitation is approximately 600-650 mm per year, being a bit higher in the southern coast areas (700-750 mm) and in the coast of Bothnian Bay and in north lower (500-600 mm). The most significant hydrological episode in Finland is the spring snowmelt usually in April-May, when approximately 50% of the annual runoff is produced within a short time period. Many wetlands (>1000) have been built in Finland to decrease agricultural nutrient loading, but for polishing purified domestic wastewaters, CWs have been rather an ignored opportunity.

1.6 MEASUREMENT OF NITROGEN REMOVAL IN AQUATIC ENVIRONMENT

Currently, the knowledge of the global amount of N_r transformed back to N_2 is poor (Durand et al. 2011; Fowler et al. 2013; Galloway et al. 2004). Studying N process rates is complicated because N is constantly transformed within several simultaneous processes (see chapter 1.4). Another highly challenging feature is the high background concentration of N_2 in the atmosphere as well in aquatic systems, which makes it difficult to quantify the relatively small N_2 production. Mass-balance studies are widely used for entire aquatic systems. They are based on the measurement of the amount of all N_r species from inlet and outlet of the system. Typically, only “N retention” is reported and it often remains a question, whether the N_r has been storaged in the system (sedimentation, assimilation) or removed (denitrification, anammox)? To have a precise estimation of denitrification with mass-balance method, all other terms in the N budget should be measured. This is subjected to errors, especially in larger systems.

Several methods have been used to quantify denitrification in aquatic environments. Earlier, the most used method for studying denitrification in sediments was acetylene inhibition technique (Sørensen 1978). It is based on the inhibition of the last reducing step in denitrification and the end product N_2O is measured which is considered to be equal with N_2 production. The advantage of this method is that measuring N_2O is fairly simple compared with the measurement of N_2 because of the lower background concentration. As the samples can be measured with gas chromatograph, AI is not expensive enabling high number of samples. The method has been found to underestimate denitrification rates due to a inhibition of nitrification leading to a decreased production of NO_3^- (Seitzinger et al. 1993; Steingruber et al. 2001). Therefore, the method is questionable in more pristine environments with low nitrate levels and environments relying on the nitrate produced through nitrification. Systems with high NO_3^- concentration this method is more suitable (Groffman et al. 2006).

Originating from terrestrial studies in the 1950s, methods applying stable isotopes of N (^{14}N, ^{15}N) began to being used in the aquatic field in the 1980s. Most (99.63%) of the N in the environment has a mass number (protons and neutrons combined in the nucleus) of 14 (^{14}N) (Clayton 2003), so N_2 molecules are found mostly with mass number 28 ($^{28}N_2$). As for the high background concentration of $^{28}N_2$, the production of this natural $^{28}N_2$ cannot be directly measured. Nielsen (1992) developed the isotope pairing technique (IPT) which has been widely applied in several aquatic studies in freshwater and marine environments (Steingruber et al. 2001). This method was designed for measuring sedimental denitrification and is based on the addition of labeled nitrate $^{15}NO_3^-$ to the water above the sediment. The production of single-labeled $^{29}N_2$ and double-labeled $^{30}N_2$ molecules is measured. High advantage of the IPT is that it can differentiate the denitrification that is based on the NO_3^- of the water column (D_w) and the denitrification of the NO_3^- produced in the sediment by nitrification (D_n). The method is based on few fundamental assumptions: 1) the homogenous mixing of the labeled $^{15}NO_3^-$ with the ambient $^{14}NO_3^-$ in the denitrification zone in the sediment (i.e. the depth where oxygen is depleted), 2) the independence of the denitrification of the ambient $^{14}NO_3^-$ (produced $^{28}N_2$) of the added $^{15}NO_3^-$ concentration, 3) the dependence of the denitrification of the labeled $^{15}NO_3^-$ rate to the added $^{15}NO_3^-$ concentration. Whether the conditions in the studied sediments meet the required assumptions, can be verified in pre-experiments with a concentration series incubation on each study site. An example of this is shown in Fig.3 from the study **I**. Sufficient amount of the label $^{15}NO_3^-$ relative to the ambient $^{14}NO_3^-$ can be clarified with the same pre-experiment. If the added label amount is too low, not so much of the $^{14}NO_3^-$ ends up in the measured N gas molecule $^{29}N_2$, and the risk of underestimation of denitrification rate is higher (Nielsen 1992).

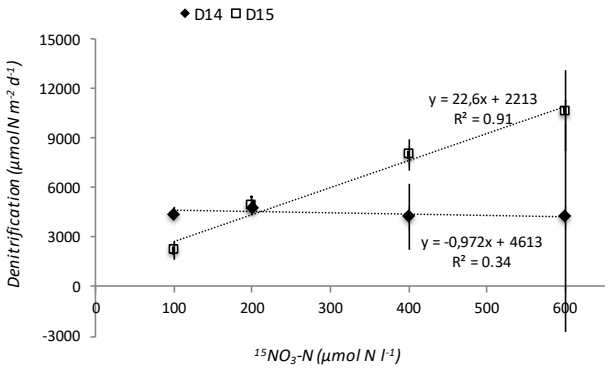

Figure 3. An example of a pre-experiment with $^{15}NO_3$-N concentration series. D14 = denitrification of the natural $^{14}NO_x$-N, D15 = denitrification based on the labeled $^{15}NO_3$-N. Standard deviation shown with error bars, n=3 for each data point. Modified from the study **I**.

The sedimental denitrification rate of ambient $^{14}NO_3^-$ (D_{14}) according to the IPT is calculated by first calculating the sum of ^{15}N in the produced N gas (D_{15}):

(1) $$D_{15} = (^{14}N^{15}N) + 2(^{15}N^{15}N)$$

D_{14} is then calculated from D_{15}:

(2) $$D_{14} = D_{15} * (^{14}N^{15}N)/(2(^{15}N^{15}N)$$

With D_{15}, it is possible to calculate the amount of sedimental denitrification originating from the water column nitrates (D_w) based on the concentrations of $^{14}NO_3^-$ and $^{15}NO_3^-$, as follows:

(3) $$D_w = D_{15} * {}^{14}NO_3^- / {}^{15}NO_3^-$$

D_n can then be calculated by subtracting the D_w from the total D_{14}. The orignal IPT by Nielsen (1992) assumes that denitrification is the only process producing N_2. With anammox, conditions fail to meet the assumption of IPT of the binomial distribution of produced N gas species, as anammox combines $^{14}NH_4^+$ with both, $^{14}NO_2^-$ and $^{15}NO_2^-$ and more $^{28}N_2$ and $^{29}N_2$ is produced than would be merely by denitrification. This causes overestimation of denitrification (Risgaard-Petersen et al. 2003). Recommended pre-experiment with the different $^{15}NO_3^-$ additions reveals the presence of anammox which would be indicated as a rising trend in the D_{14} rates relative to label additions. If anammox accounts only a small part of N_2 production, the overestimation is not serious and the process might not be shown as a rising rising trend in D_{14}. Trimmer et al. (2006) developed a technique based on direct measument of labeled $^{15}N_2O$ in addition to produced N_2 from each incubated core. As anammox does not produce N_2O as denitrification does, the contribution of denitrification and anammox on each incubated sediment core

can be calculated. This is also important considering the potential heterogenity in sediments.

Many of the studies on shallow wetland and stream sediment denitrification are conducted in dark laboratory at steady temperature (e.g. Castaldelli et al. 2015; Pinardi et al. 2009; Roach & Grimm 2011). The conditions may not represent the true situation, *in situ* in the field, and might result in over- or underestimations. Furthermore, most of the studies are done in the summertime and samples collected during the daylight. In the boreal cold region with high seasonality, more information of denitrification rates is needed to clarify the real potential of shallow wetlands to remove excess N.

1.7 MEASUREMENT OF AQUATIC INORGANIC NITROGEN LOADING

The need for reliable measurement of N loading concerns especially non-point sources like agricultural areas which are the main source of inorganic N i.e. nitrate in Finland (Lepistö et al. 2006). N loading of the watershed is calculated as the sum of discharge of a stream or a river (l h^{-1}) and N concentration (mg N l^{-1}). N loading is affected by air and soil temperature, precipitation and vegetation, but also by human practices in the watershed like fertilization and land use. Point sources, like WWTPs and other industrial premises, are often required to monitor their nutrient loading and so their effect on the environment are relatively well known. Non-point sources in the boreal region release most of the nutrient loading from watersheds outside the growing season and in addition to spring snowmelt, few heavy rain events can have a major contribution to the yearly load (e.g. Puustinen et al. 2007). Traditionally, nutrient loading calculations have been based on sporadic sampling, samples taken biweekly for example. This type of nutrient load monitoring may miss the high N peaks. Continuous, high-frequency measurements conducted with optical sensors, can be used to get more reliable load estimates. Agricultural practises in the catchment may be observed as elevated N concentrations only for a short period of time simultaneously with higher discharge. In highly dynamic small stream waters, the contribution of relatively short N peaks can be significant to the yearly loading and thus, load estimates are subject to errors (e.g. Linjama et al. 2009; Koskiaho et al. 2010) with sporadic sampling. In addition to load estimates, continuous monitoring can be useful in comparing different nutrient mitigation strategies performed in the catchment and measuring the performance of a CW as a nutrient reduction strategy (Valkama 2018; Wahlroos et al. 2015).

In the boreal region, humic brown-coloured waters are common and organic matter in aquatic systems is leached from the catchments (Kortelainen et al. 2013). In Finland, 86% of the land area is covered with forests, mainly Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*). Peatlands cover

approximately 1/3 of the land (FFRI 2014). Average TOC in surface waters varies between 0.5 and 47.0 mg l^{-1} with a median of 12.0 mg l^{-1} (Kortelainen 1999). Typically highest OC concentrations are found in small and shallow lakes of central and southern Finland due to the higher primary production relative to the northern parts of the country (Kortelainen et al. 2004). In addition to the quantity, the quality of OC varies. Increasing share of peatlands and forests in the watershed has been found to be connected to a decreasing amount of bioavailable DOC and more agricultural area was found to increase the C bioavailability and the amount of DON (Asmala 2014).

Optical sensors measuring agricultural excess N consisting mostly of NO_3-N, have been used in freshwater studies mainly during the last 10-15 years (Pellerin and Bergamaschi 2014). The gerenal principle in the use of optical sensors is the measurement of incident light in the water. Substances absorb light at known wavelengths and in transmittance measurements, the concentration of the substance in question is inversely proportional to the transmitted light amount. Optical sensors for NO_x-N measurements cannot be used completely without traditional water samples because they need to be calibrated in the measured water matrix to get reliable results. In calibration, the sensor result is compared with the reference method i.e. the laboratory-studied water sample. Calibration separates the sensor signal from interfering substances with partially similar absorbance properties with NO_x^--N, like organic carbon (OC) (Pellerin et al. 2013). In addition, turbidity causes scattering and shading throughout the spectrum. Sufficient maintenance and cleaning is also important in avoidance of fouling of micro-organisms, etc. as well as chemical precipitates. If the correlation between laboratory NO_x^--N results and measured NO_x^--N concentrations with optical sensor is weak, the possible interference from OC and should be checked.

1.8 AIMS OF THIS THESIS

The aims of this thesis are focused on N removal and retention in boreal constructed wetlands and the use of optical sensors in the measurement of inorganic N. Constructed wetlands are generally regarded as efficient sites for N retention. However, the amount of N being removed i.e. converted to N_2 and N_2O is unclear, especially when wetlands in the cold climate are considered. This thesis aims to answer to these three study questions:

1. Which environmental factors govern the main nitrogen removal process, sedimental denitrification rate, in a shallow agricultural boreal wetland? (**I**)

2. Are constructed wetlands applicable in mitigating high nitrogen loading from wastewater treatment plant in a cold climate? (**II**)

3. Can optical sensors be applied in nitrate measurements reliably concerning the potential interference from organic carbon? (**III**)

No mud. No lotus.

2 MATERIALS & METHODS

2.1 STUDY SITES

2.1.1 KOIRANSUOLENOJA CATCHMENT (I, III)

Denitrification and regulating environmental factors were studied in a small wetland in an agricultural watershed located in southern Finland. Brook Koiransuolenoja (Fig. 4) collects waters from a small watershed (6,8 km^2) mostly covered with coniferous forest (71%). The soil is easily erodible (Tikkanen et al. 1985) and 24% of the catchment area is cultivated (Arvola et al. 2015). As a result, the turbidity is highly variable in the brook water. During the study the flow was was ca. 60 l s^{-1} and the average theoretical retention time one hour (0.25-4.5 h). In 2013, a chain of three sedimentation ponds were built by the brook. One of the constructed ponds (area of 320 m^2, volume of 226 m^3) was selected as a study wetland because of the high NO_x^--N concentration (ca. 2.7 ±0.81 mg N l^{-1}) and the shallow littoral (littoral depth 0.3-0.4 m, mean wetland depth 0.7 m).

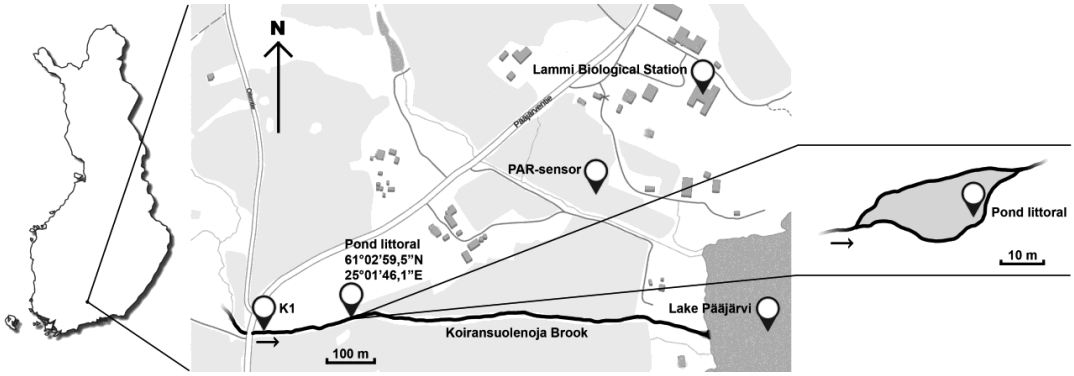

Figure 4. Location of Brook Koiransuolenoja and the study wetland. Monitoring site of the brook water (K1) is situated upstream from the pond. The location of the sensor for measuring photosynthetically active radiation (PAR) and laboratory of Lammi Biological Station.

2.1.2 CONSTRUCTED WETLAND OF LAMMI WASTEWATER TREATMENT PLANT (II)

The CW of Lammi WWTP was chosen as a study site as it offers a possibility to investigate nutrient retention and N removal efficiency in a cold climate. The Lammi CW (61°08′83′′N, 24°99′66′′E) was originally constructed in 1963 for treating the wastewaters of a local dairy. From the 1970s, the square shaped 4ha-wetland (mean depth 1.0 m; Fig. 5) has been used as a polishing step in municipal wastewater treatment during the colder seasons. The use of the CW in warm season has been avoided because of the high production of algae biomass that could have been considered as unpleasant and alarming when discharged to the recipient Lake Ormajärvi. Consequently, the purified wastewater was directed straight to the lake during warm season. Since the beginning of the study (**II**), the CW has been used in nutrient removal year round. Today, the WWTP (Hämeenlinnan Seudun Vesi Ltd.) treats the wastewaters of ca. 4000 people with sand separation, P removal and nitrification before the water is discharged (avg. 871 m^{-3} d^{-1}) into the wetland. Most of the N load coming from the WWTP is in the form of NO_x-N (67%), and the average concentration in the water flowing into the CW is ca. 20 mg NO_x^--N L^{-1}. Approximately ¼ of the N load is discharged into the wetland as NH_4^+-N.

Figure 5. Constructed wetland of Lammi wastewater treatment plant, Hämeenlinnan Seudun Vesi Ltd.

2.1.3 SURFACE WATERS WITH DIFFERING NITROGEN AND ORGANIC CARBON CONCENTRATIONS (III)

In addition to Brook Koiransuolenoja (section 2.1.1.), the waters of eight other sites, lake, river, brook, spring, with varying NO_x-N and OC concentrations in southern Finland were included to study the use of continuous optical sensors in monitoring N concentrations (**III**). Table 2. shows the NO_x^--N and OC concentrations during the study. Brook Laavionsuonoja is a small stream flowing from a peatland area and has a high OC content.

Table 2. *Sampling sites with NO_x^--N and DOC or/and TOC concentrations in the studied waters.*

Site	Coordinates	NO_x-N mg l^{-1}	DOC mg l^{-1}	TOC mg l^{-1}
Brook Laaviosuonoja	61°02'N, 24°59'S	0.040 *(±0.010)*	74.0 *(±0.38)*	53.0 *(±0.61)*
Lake Ormajärvi	61°05'N, 24°57'S	0.35 *(±0.010)*	nd	7.8 *(±0.030)*
Spring Löytynlähde	61°02'N, 24°58'S	0.41 *(±0.010)*	1.2 *(±0.29)*	2.3 *(±0.060)*
River Luhtaanmäenjoki	60°20'N, 24°47'S	0.57	7.6 *(±0.037)*	nd
Lake Pääjärvi	61°05'N, 25°05'S	0.85	10.0 *(±0.038)*	nd
River Teuronjoki	61°05'N, 24°50'S	1.2	8.1 *(±0.033)*	nd
River Vantaanjoki	60°40'N, 24°56'S	1.3 *(±0.012)*	nd	10.0 *(±0.24)*
Brook Letkunoja	61°03'N, 25°05'S	2.5	7.9 *(±0.028)*	nd
Brook Koiransuolenoja	61°03'N, 25°04'S	2.4 *(±0.37)*	10.6 *(±4.5)*	nd

2.2 MEASUREMENTS AND ANALYSES

2.2.1 WATER, SEDIMENT AND ENVIRONMENTAL VARIABLES

Photosynthetically active radiation (PAR) was measured with a quantum sensor (PQS1, Kipp & Zonen, Germany) every 10-minutes, and the sensor was located close (600 m) to the study site (**I**). Air temperature and precipitation data were obtained from The Finnish Meteorological Institute (**I**). Water flow and sediment temperature were measured with a flow meter (MiniAir2, Schiltknecht, Germany) during each sampling occasion (**I**). Water temperature and dissolved oxygen saturation and concentration of the water were measured with oxygen meter (ProODO, YSI, USA) (**II**). O_2 concentration in the water close to the sediment surface, as well as O_2 penetration depth (OPD) in the sediment, were measured with microelectrode (Unisense OX100, Denmark) connected to a picoammeter (PA2000, Unisense, Denmark) (**I**). Sediment samples were collected by hand (**I-II**) or with sediment sampler (**II**). For LOI analysis, top sediment (0-3 cm) was combusted at 550 ºC for 2h. Water samples for nutrient, carbon and chlorophyll *a* (Chl) analysis were collected by hand (**I-III**) and analysed in the laboratory of Lammi Biological Station.

For dissolved inorganic nutrient fractions and dissolved organic carbon analysis the samples were filtered through 0.45 µm filters (Millex-HA, Merck Millipore, USA) (**I-II**). TN, NO_x^--N, NH_4^+-N, TP and PO_4^{3-}-P concentrations were analyzed spectrophotometrically (Gallery Plus, Thermo Scientific, Finland) according to standards SFS-EN ISO 11905-1, SFS-EN ISO 13395, SFS-EN ISO 11732, ISO/DIS 15681-2 respectively (**I-II**). DOC was analyzed using a carbon analyzer (Ordior TOC-V, Shimadzu, Japan) according to standard SFS-EN 1484 (**I-II**). In study **III**, water samples were filtered (<0.2 µm, Nuclepore, Whatman GE Healthcare, USA) for NO_x^--N and DOC analysis. NO_x^--N was analyzed with an automated flow-injection analyzer (Lachat QuikChem 8000, Lachat Instruments, USA), and TOC and DOC were analyzed using a high-temperature combustion method (TOC-5000, Shimadzu, Japan) (**III**). For Chl *a*-analysis, the water was filtered (Whatman GF/C glass-fiber, UK) and filters were freezed (-20 °C,) before spectrophotometrical analysis (UV-1800, Shimadzu, Japan) according to standard SFS 5772 (**I-II**).

2.2.2 SEDIMENTAL NITROGEN REMOVAL MEASUREMENTS AND ANALYSES

For denitrification measurements intact sediment samples were collected by hand (**I-II**) or with sediment sampler (**II**). Denitrification rates were measured using the isotope pairing technique (IPT) (Nielsen 1992, Risgaard-Petersen et al. 2003) (**I-II**), which is described earlier in section 1.6. Porosity of the studied sediment was calculated from top sediment (0-3 cm).

In the agricultural wetland, N_2-production was studied all year round using labeled sediment cores (n=3) incubated in ambient temperature and light conditions in the field using one label concentration (200 µmol $K^{15}NO_3$) (total n=68) (**I**). The average NO_x^--N concentration in the agricultural brook was 194 µmol N l^{-1} (2.7 mg N l^{-1}). In addition to seasonal variation, diurnal variation was investigated twice in a 24-hour experiment in August 2014 and 2015 (**I**). Validity of the method in the studied sediment was verified using concentration series incubations with ^{15}N–labeled potassium nitrate (50, 100, 200, 400, and 600 µmol of $^{15}NO_3^-$-N, 98 atom%, Sigma-Aldrich).

In the WWTP wetland (**II**), N_2-production was measured twice using a $^{15}NO_3^-$ -concentration series in the non-vegetated and vegetated area during the summer. The average NO_x^--N concentration flowing from the WWTP in to the CW was 1400 µmol N l^{-1} (19.6 mg N l^{-1}). The NO_x^--N concentration was assumed to be higher in the non-vegetated area than the vegetated area. Concentrations of $K^{15}NO_3^-$-N: 150, 350, 700 and 1000 µmol were used in IPT incubations in the vegetated area cores, and 400, 1100, 1800 and 2500 µmol in the non-vegetated area. In cold season, N_2-production by denitrification and anammox, as well as N_2O-production, were measured with a series of 12

label concentrations between 1700 and 3825 µmol $K^{15}NO_3^-$-N. Ambient NO_x^--N concentration at the time was 1780 µmol (24.9 mg N l^{-1}). Nitrogen isotope mass areas (*m/z* 28, 29, 30, 44, 45, and 46), and N_2 and N_2O concentrations were analyzed with isotope ratio mass spectrometer (CF-IRMS, Isoprime Ltd, UK).

2.2.3 MEASUREMENT OF NITROGEN RETENTION IN WWTP WETLAND

We assumed the same amount of water enters the wetland that is delivered to the WWTP on daily basis (**II**). Wetland nutrient retention (%) or release was calculated as the difference between load in to the wetland and load out relative to the incoming load. Therefore, retention refers to the loss of all N fractions by also other than microbial meditated processes, and removal refers only to N_2 and N_2O production as described in the previous section.

2.2.4 SETUP OF THE NITRATE MEASUREMENTS WITH OPTICAL HIGH-FREQUENCY SENSORS

A variety of different types of surface waters were studied with a high-frequency optical UV/Vis sensors (Messtechnik, Austria) using 35-mm and 5-mm measuring pathlengths (MP) (**III**). These sensors are able to measure NO_x^--N, DOC, TOC and turbidity. According to the manufacturer, the sensor with the longer measuring pathlength (MP) can be used for measuring the lower NO_x^--N concentrations (<10 mg N l^{-1}) and the sensor with the shorter MP, NO_x^--N concentrations of 10-70 mg N l^{-1} can be measured. Similarly, the sensor with longer MP can be applied measuring lower OC concentrations (TOC <25 and DOC <12 mg C l^{-1}) and the one with shorter MP can measure the higher concentrations (TOC <150 and DOC <90 mg C l^{-1}).

The results by the sensors were compared with the laboratory results in three experiments: 1) concentration of different types of waters were measured, and also when diluted with deionized water as well as when mixed with each other (Table 2) with both 35-mm and 5-mm MP sensors, 2) spring and peatland waters were spiked with different additions of potassium-nitrate (measured only with 5-mm MP), 3) 5-mm MP sensor was applied in Brook Koiransuolenoja at site K1 (Fig. 4) measuring NO_x^--N and DOC in in every 30 minutes for 6 months. The sensor NO_x^--N results were compared with NO_x^--N samples analyzed in laboratory. Two different calibration methods were used for correcting the sensor NO_x^--N results.

2.3 STATISTICAL ANALYSIS

I: The relationships between environmental factors and seasonal denitrification were investigated with correlation, head component analysis, and structural equation modeling (SEM, e.g. Sutton-Grier et al. 2010). The differences in denitrification and environmental factors during light and dark in the diel data were tested with a paired t-test and nonparametric Wilcoxon rank test. General linear model (GLM) was used to explore the relationships between denitrification rates and environmental variables.

II: The differences in nutrient retention between the study years and between ice-free/ice-cover periods were tested with nonparametric Wilcoxon rank tests. Linear regression analysis was used to investigate the relationships between environmental factors and nutrient retention/chlorophyll *a*.

III: The differences between the laboratory and the sensor NO_x^--N results were compared using simple linear regression (SLR) and the accuracy of the sensor was evaluated using the correlation coefficient (R^2). The differences between the results of the two methods were investigated with nonparametric Wilcoxon rank test. The effect of increasing DOC concentration on the NO_x^--N sensor result was evaluated by the proportional difference between the laboratory and sensor results. The accuracy of the two calibration methods, SLR and multiple linear regression (MLR), was evaluated and tested by linear regression and nonparametric Wilcoxon rank test.

3 RESULTS & DISCUSSION

3.1 NITROGEN REMOVAL (I-II)

3.1.1 DENITRIFICATION IN AGRICULTURAL WETLAND (I)

Denitrification rates (N_2) in the seasonal measurements in the agricultural wetland (**I**) varied from 0 to 174 mg N m^{-2} d^{-1} (Fig. 7) being 34.2 mg N m^{-2} d^{-1} in average. As compared to my results, similar or higher rates have been measured from nitrate-rich systems from temperate region (e.g. de Klein 2008; Racchetti et al. 2011; Soana et al. 2017) while rates from boreal lakes have found to be substantially lower (Table 3). Denitrification was governed mainly by temperature (Fig. 6) followed by oxygen and turbidity. The sediment surface temperature in the studied littoral stayed above the freezing point (+0.1–17.2 °C) during the seasonal study and was higher than air temperature in winter. Average OPD in the littoral was 9.5 mm (2.5–18.0 mm) and average LOI of the upper sediment in the wetland littoral varied between 7.3–9.8%. Average stream turbidity was 13.5 ftu (1.8–36.3 ftu) and DOC concentration 9.9 mg C l^{-1} (5.9–17.1 mg C l^{-1}).

Denitrification rates are considered to be very slow at temperatures below 5 °C (e.g. Bremner & Shaw 1958). At the minimum sediment surface temperature of +0.1 °C, no denitrification was detected, but at +0.4 °C, the rate was 1.8 mg N m^{-2} d^{-1}. At the time of the snowmelt in March, denitrification rate was 15.9 mg N m^{-2} d^{-1} despite the low sediment temperature (+0.7 °C). This rate was similar with the ones measured in September at higher temperatures (avg +9.4 °C). At the time, we also measured the maximum NO_x^--N concentration in the wetland littoral (5.2 mg NO_x^--N l^{-1}). Previously, denitrification has been measured under such low temperatures (<2 °C) only in a few boreal studies (Table 3). Some studies have found denitrification rates correlating negatively with temperature while positively with NO_x^--N availability during winter (Aalto et al. 2018; Rissanen et al. 2011). It seems that the high NO_x^--N concentration may, to some extent, counteract the limiting effect of declining temperature, which is in line with some studies where NO_x^--N concentration has been the most important factor governing N removal by denitrification (e.g. Piña-Ochoa & Álvarez-Cobelas 2006; McCrackin & Elser 2010).

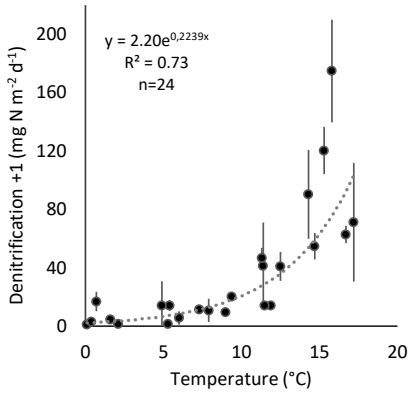

Figure 6. Relationship between denitrification rate (mg N m^{-2} d^{-1}) and the sediment surface temperature in the agricultural wetland littoral (modified from the study **I**). Denitrification rates are added with 1 mg N m^{-2} d^{-1} to remove the zero.

With the sediment temperature being the main factor governing the denitrification rates and the springtime sediment temperature staying below +10 ºC, the removal of high N load from Finnish agricultural catchments may be limited in the springtime. Furthermore, high discharge leads to short retention times in small wetlands hindering springtime N removal even more. Therefore, the suggested effective ratios between wetland and watershed area (0.5%: Hammer 1992; 1%: Crumpton et al. 1993; 2%: Puustinen et al. 2007, respectively), and the recommended water retention times, counted in days rather than hours (Leonardson 1994; Puustinen et al. 2007), should be taken into account in wetland design.

Daytime denitrification measurements may underestimate the N removal potential of shallow wetlands. In dark, the average denitrification rates were approximately 3-fold (2.5 mg N h^{-1}) compared with the average daylight rates (0.9 mg N h^{-1}). Also other studies have reported higher denitrification in dark (Christensen et al. 1990; de Klein 2008; Soana et al. 2017). Top sediment oxygenation decreased the denitrification rates and D_w was lower when OPD was higher, as the NO_x^--N had to diffuse deeper to reach the denitrification zone agreeing with the earlier studies from sediments (Andersen et al. 1984; Christensen et al. 1990; Risgaard-Petersen et al. 1994). The measured seasonal (daytime) denitrification rates were corrected using high-frequency PAR data (photosynthetically active radiation) to provide estimates for real daily denitrification. In spring and in summer the N removal may be approximately 31%, and 35% higher than when measured only during daytime. In fall, denitrification was coupled more to sedimental nitrification than to the water column NO_x^--N, suggesting the corrected denitrification rates being overestimated. The same applies to systems where denitrification is mainly based on D_n.

The importance of turbidity in regulating oxygen conditions in the sediment surface and denitrification (**I**) reflects the high importance of C for denitrification. The highest denitrification rate in the agricultural wetland (**I**)

was measured under the lowest NO_x^--N concentration (1.3 mg NO_x^--N l^{-1}) and maximum NH_4^+-N (0.5 mg NH_4^+-N l^{-1}) in July. The day before denitrification measurements, liquid manure had been spread on the fields, and was washed out into the stream by heavy rainfall. However, since the denitrification was based mainly on water column NO_x^--N (D_w% 81), the high rate cannot be explained by high NH_4^+-N fuelling coupled nitrification-denitrification. At the time, the C/N ratio was 14.0 (avg 5.1, DOC/NO_x^--N) and ON concentration 0.9 mg N l^{-1} (avg 0.4 mg N l^{-1}) in the stream water, as well as LOI% in the studied littoral, were the highest measured during the study, suggesting that availability of OC could have driven denitrification. In systems with high anthropogenic N load C may potentially limit denitrification.

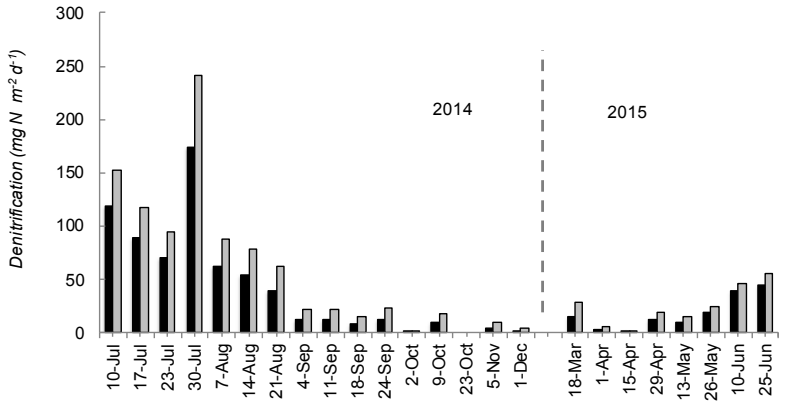

Figure 7. Measured (black columns) and estimated (grey columns) denitrification rates in Koiransuolenoja wetland littoral, modified from the study **I**.

Wetlands receiving high amounts of tile drainage water have generally low C:N ratio (1-2), whereas wetlands receiving surface flow have generally higher C:N (Grebliunas 2015; Grebliunas & Perry 2016). Not only C quantity, but the quality of C is important for denitrification (e.g. Stelzer et al. 2014). Usually microbial growth is higher in nutrient-rich waters with freshly produced C compared with oligotrophic and humic systems, where the C is less labile (Tulonen et al. 2000). In addition to tile water drainage, the study wetland (**I**) receives waters from the catchment area, which is dominated with coniferous forest. This suggests that in addition to the higher C availability (high C:N, LOI%), the C may have been more biodegradable in the liquid manure at the time of the peaked denitrification compared with the C usually discharged from the area. The lower C lability of the Koiransuolenoja wetland water compared with the Lammi CW was confirmed with measurements of $SUVA_{254}$ (DOC-specific absorbance at 254 nm) and $S_{275-295}$ (absorption slope coefficient between 275–295 nm) (Uusheimo, unpublished results).

3.1.2 NITROGEN REMOVAL IN WWTP WETLAND (II)

High denitrification rates were found in the Lammi WWTP wetland (**II**) (Table 3.). The wetland had a high growth of microalgae biomass providing fresh and labile C source. Together with reasonable temperatures in winter and high N availability, denitrification was well supported (e.g. Asmala 2014; Hietanen & Kuparinen 2008; Grebliunas and Perry 2016; Stelzer & Scott 2014). Compared with other studies conducted in CWs or lakes under low temperatures, the rates were high or similar (Table 3). The C:N ratio in the Lammi WWTP wetland was lower (avg 1.6, inlet and outlet) compared with Koiransuolenoja wetland. Low C:N ratio might be one potential explanation to the high relative amount of N_2O produced (20-46%) by incomplete denitrification during the winter measurement (**II**) (Tiedje 1988; Lu et al. 2009). Another theory for nitrate-rich systems refers that denitrifying bacteria might prefer reduction of NO_2^- to N_2O instead of reduction of N_2O to N_2, because they gain more energy from the earlier reduction step (Schlesinger & Bernhardt 2013). In addition, the enzymes mediating the denitrification process are increasingly sensitive to the presence of O_2 (Körner & Zumft 1989).

Table 3. *Mean denitrification and anammox rates (**I,II**) and results from other studies conducted under low air and water temperatures, water NO_x^--N concentrations also shown. Standard deviation (±) and minimum/maximum between brackets. *=measured with static chamber technique, ** air temperature, ***=measured from the wetland inlet and outlet.*

Study site	**Denitrification N_2** (mg N m^{-2} d^{-1})	**Denitrification N_2O** (mg N m^{-2} d^{-1})	**Anammox** (mg N m^{-2} d^{-1})	**Temperature** (ºC)	**NO_x^--N** (mg N l^{-1})	**Reference**
Finland, CW polishing municipal wastewater	117 ±49 (67-252)	44 ±23 (18-96)	8 ±7 (0.4-20)	12.2 (3.0-18)	8.6 ±9.3 (2.4-24.9)	Uusheimo et al. 2018 (**II**)
Finland, agricultural CW	34 ±43 (0-174)	-	-	8.9 ±5.5 (0.1-17.2)	2.3 ±1.0 (1.4-5.2)	Uusheimo et al. 2018 (**I**)
Finland, shallow lake	4.5-5.6	0.8-0.9	-	2.6-15.5	0.23-1.4	Aalto et al. 2018
Finland, shallow lake	3.0-5.8	-	-	0.8-20.5	0-0.7	Holmroos et al. 2012
Finland, shallow lake	0.6-8.6	-	-	0.5-21.3	0.04-0.5	Rissanen et al. 2011
Finland, petland CW polishing municipal wastewater	-	0.2-16*	-	2.9-26**	4.6-7.0***	Karjalainen et al. 2005
US, Illinois, agricultural CW	48-283	-	-	4.0-25.0	4.3-10.5	Xue et a. 1999

Incoming C:N ratio (avg 1.2) was lower than the ratio in wetland outlet (avg 3.0). In July and November 2016, the two peaks in the incoming C:N resulted from a very low NO_x^--N concentration (<6 mg N l^{-1}, avg 20 mg N l^{-1}) discharged from the WWTP (Fig. 8). Simultaneously in November, the DOC concentration was high. The reason for low NO_x^--N in July was the sudden massive increase in wastewater volume originating from a Scoutcamp of 10 000 people, resulting in disturbance of the nitrification process. During both study years in November, the NH_4^+-N load relative to NO_x^--N load increased suggesting the nitrifying microbes in the WWTP were suffering from a coldschock, but were able to acclimatize gradually during the winter. This is indicated by the increased relative share of NO_x^--N discharged to the wetland.

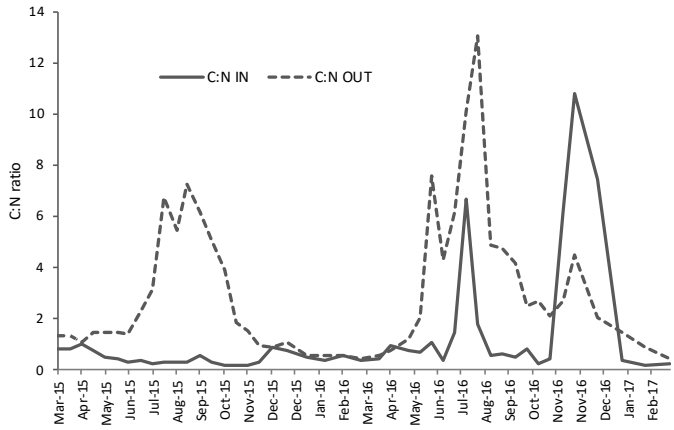

Figure 8. The variation of the C:N ratio in the wastewater treatment plant discharge into the wetland as well as in the wetland outlet.

The D_w dominated denitrification in the vegetated littoral sediment of the WWTP CW, even though the bottom was covered with *Typha* litter growing by the shore. The roots of wetland vegetation have been found to support D_n by delivering O_2 into the deeper sediment creating microsites with anoxic/oxic interfaces (e.g. Reddy et al. 1989). However, *Typha* is also able to assimilate NH_4^+-N in addition to NO_x^--N and competes with the substrate with nitrifiers (Brix et al. 2002).

3.2 NITROGEN RETENTION (II)

Most of the N discharged into the Lammi CW was in the form of NO_x^--N (67%) and appr. 1/4 was as NH_4^+-N. In average, the CW retained most of the N loading (Table 4). During ice-free periods, a large portion of the N load was transformed into PON through the growth of microalgae, and the amount of

PN and PP increased with Chl *a* (R^2= 0.83 and 0.84, respectively). Based on the measured denitrification rates in summer and winter, denitrification was responsible for most of the measured NO_x^--N retention (72%) as has been suggested previously (Vymazal 2007; Xue et al. 1999; Lin et al. 2007). Retention time and temperature are considered as the key factors for efficient N removal in the wetlands of the cold climate (Ronkanen & Kløve 2009).

In addition to the long retention time (ca. 41 days) and high denitrification rates (Table 3), the efficient N retention in the CW can be explained by efficient coupled nitrification and denitrification removing both NH_4^+-N and NO_x^--N. Indeed, the results indicate that the wetland can support anoxic as well as oxic N processes. Spatial variability is considered being one of the most important factors for efficient N removal in CWs receiving raw or treated wastewaters (i.e. treatment wetlands) with variable NH_4^+-N/NO_x^--N loads (Brix 1993; Vymazal 2010). It is achieved by creating different microenvironments within the wetland, e.g. sequential wetland basins with differing oxygen conditions. Nitrification in wetlands has been found to be most active in oxic surfaces in the water column. For example, the highest nitrification potential in a boreal treatment wetland was found on twigs, followed by sediment and plants (Kallner Bastviken et al. 2003). Generally, vegetation is considered beneficial for N removal as it provides surface area for the microbes in the oxic water column as well as carbon as an energy source for denitrifiers in the sediment through root exudates (Henry et al. 2008).

Table 4. *Total nutrient retention (%) in the study* ***II****. Minus indicates that more of the compound in question is being produced in the wetland than it receives from the wastewater treatment plant.*

3/2015-3/2017	**DOC**	**NH_4-N**	**PO_4-P**	**NO_x-N**	**TN**	**TP**	**PN**	**PP**
Ice-free periods (IV–XI)	-4.9	81.3	88.8	71.0	61.2	10.1	-202.5	-14.4
Ice-cover periods (XII–III)	-0.5	8.2	-3.8	37.7	28.2	34.5	0.7	46.2
Total	**-3.8**	**60.8**	**68.0**	**61.5**	**51.6**	**15.6**	**-121.2**	**-0.7**

In the 2-year study (**II**), mean N retention differed between ice-cover and ice-free periods ($p<0.05$), being lower in ice-cover periods. In the ice-free periods, the retention was always positive (75% and 83% for NH_4^+-N, 74% and 68% for NO_x^--N, 63% and 61% for TN, first and second year, respectively). During ice-cover, the retention was 49% and -54% for NH_4^+-N, 19% and 52% for NO_x^--N and 24% and 32% for TN, first and second year, respectively. The change in the NH_4^+-N and the NO_x^--N concentration between the wetland outlet and inlet shows how the CW mitigates the NH_4^+-N loading during the second study year to the recipient lake (Fig. 9). The occasional NH_4^+-N release

during ice-cover periods can be explained by the lower O_2 availability for nitrification compared with ice-free period (Fig. 10). Similar results have been observed in a natural peatland polishing treated wastewater in northern Finland where treatment wetland successfully removed 20-52% of NO_x^--N load. The NH_4^+-N removal was low due to a limited nitrification under low O_2 availability (Karjalainen 2016). One possible explanation for promoting lower O_2 saturation and the release of NH_4^+-N and PO_4-P during the second ice-cover period could be the high algae biomass produced between May and November (avg 438, max 1044 Chl µg l^{-1}) in the second study year. In conclusion, it is recommended to use oxygenation during ice-cover to prevent the release of NH_4^+-N and PO_4-P in the CW.

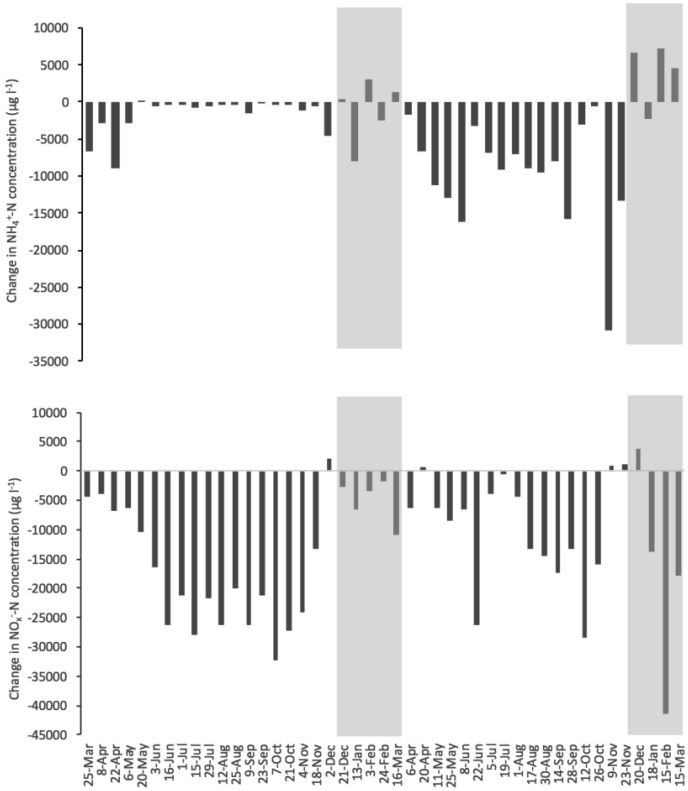

Figure 9. Average change in NH_4^+-N concentration between Lammi CW outlet and inlet (**II**). Negative change indicates NH_4^+-N retention, positive change NH_4^+-N release. Ice-cover periods marked with grey areas.

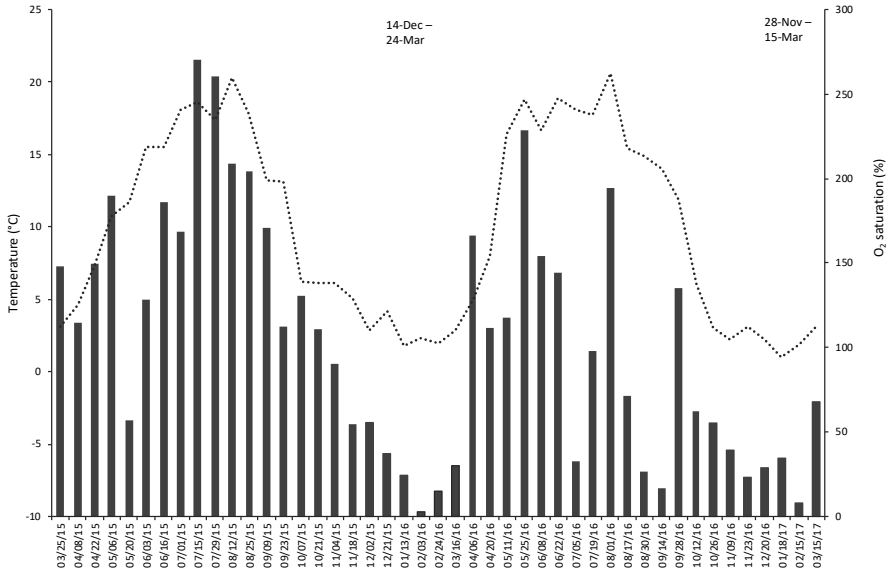

Figure 10. Temperature (line) and oxygen saturation (columns) in the WWTP wetland discharge between 25 March 2015 and 15 March 2017. Ice-cover periods are indicated with gray areas (**II**).

Results of nutrient retention from different kinds of northern CWs (Table 5) are mostly positive. Negative results have been reported mainly from small wetlands relative to the watershed area (<0.5%) (e.g. Koskiaho 2006; Vuollekoski et al. 2015). Comparable results with the Lammi WWTP are not available from Finland. In the study **I**, we did not measure N retention based on mass balance, as the difference between the incoming and outflowing NO_x^--N in the small agricultural wetland most likely would have not been sufficient to be detected in the laboratory analyses during other seasons than summer. Microbial N processes have generally high spatial and temporal variability (e.g. Rissanen et al. 2011; Hernandez & Mitch 2007; Kallner Bastviken 2003) and the highest denitrification rates where found in the deepest part of the Koiransuolenoja wetland being nearly 2-fold compared with the shallow littoral (Uusheimo, unpublished results). In addition to denitrification, other processes (sedimentation, assimilation) affect to overall N retention. N retention estimate for the entire Koiransuolenoja wetland based merely on littoral denitrification rates presented in Table 5. may be an underestimation.

Table 5. *Average total nitrogen retention rates in constructed wetlands treating agricultural or municipal wastewaters in cold climate.*

CW location, wastewater type	**Wetland size (ha)**	**Nitrogen retention (g N m^{-2} y^{-1})**	**Reference**
Sweden, agricultural	180	43 - 46	Leonardson et al. 1994
Sweden, agricultural	0.22 – 2.1	1.7 - 152	Strand & Weisner 2013
Sweden, municipal	26	146	Svedin et al. 2008
Sweden, municipal	20 - 28	70 – 150	Andersson et al. 2005
Norway, agricultural	0.035 - 0.09	50-285	Braskerud 2002
Finland, agricultural	0.5 - 0.6	-52 - 28	Koskiaho 2006
Finland, agricultural/urban	0.5	18	Valkama et al. 2017
Finland, municipal	4.0	108	Uusheimo et al. 2018 (**II**)
Finland, agricultural	0.032	12.6	Uusheimo et al. 2018 (**I**)*
USA, Lake Michigan, natural peatland, municipal	100	4.4	Kadlec 2009
Mean value from 203 CWs, mainly boreal and temperate region	-	184	Land et al. 2016

*based only on littoral denitrification estimates from study (**I**).

As denitrification rates generally correlate positively with water NO_x^--N concentration, N_2O emissions are greater in CWs treating sewage compared with more pristine wetlands (Karjalainen et al. 2005; Søvik & Kløve 2007). CWs of North Europe are not considered as significant sources of N_2O emissions because of their relatively small surface areas (Søvik et al. 2006; Karjalainen 2016). N_2O emissions of conventional wastewater treatment plants may be much higher than of CWs treating or polishing wastewater (Czepiel et al. 1995, Hanaki et al. 1992). In the winter measurements of Lammi CW, the share of N_2O from the total denitrification was notable (20-46%) and the potential explanation arising from low C:N ratio was presented in section 3.1.2. However, it is not possible evaluate the annual N_2O production reliably with only one N_2O measurement and the issue does need further investigations. Low temperature has been found to affect nitrous oxide reductase more than enzymes producing nitrous oxide (Holtan-Hartwig et al. 2002). Moreover, the winter measurement was conducted in sediments from the non-vegetated area with lower OC amount (LOI%) compared with the littoral vegetated sediment. Denitrying microbes have been reported to be stimulated by the amount of root exudates. In addition, the quality of the root exudates was reported to affect the share of produced N_2O of total denitrification (Henry et al. 2008). However, in nitrate-rich systems, the root exudates alone would not be sufficient to maintain high N removal (Zhai et al. 2013). Denitrifying bioreactors with different solid C substrates, for example woodchips, have been suggested as an enhancement strategy to treat agricultural waters with low C:N (Schipper et al. 2010).

Given that low temperature does decrease the general N removal rates and the retention efficiency of CWs, few solutions have been presented to overcome at least to some extent the challenge arising from the cold climate. Higher hydraulic retention time can partially compensate the low N removal rates in cold climate, as the water has a longer contact time with sediment (Addy et al. 2016). In CWs where water table is below the soil surface, the soil is exposed to freezing air temperatures, and do require some type of insulating cover (e.g. leaf litter, mulch). In free water surface CWs of cold climate like in Finland, ice formation and snow cover both provide significant and natural insulation approximately for the coldest 4 months (Kadlec & Wallace 2009).

3.3 MONITORING NITRATE CONCENTRATIONS WITH OPTICAL SENSORS (III)

Typically the highest diffuse DIN concentrations are measured in agricultural watersheds consisting mostly of NO_x^--N, whereas in forested areas and peatlands the N load originates mainly as organic form. This was shown also in study **III**, as the lowest NO_x^--N concentrations were found in the bog outlet and the highest concentrations in the brook and river waters draining from the agriculturally influenced catchments. The lowest OC concentration was in the spring water and the highest in bog outlet. As the optical sensors measure TOC and DOC in addition to NO_x^--N, it was first investigated how the sensor results correlate with the reference method, i.e. laboratory TOC and DOC results. A strong correlation was found with both sensors: 35-mm sensor R^2=0.91 (n=41) and 5-mm sensor R^2=0.97 (n=51) as compared with the reference method.

Organic carbon concentration generally increased the NO_x^--N measured with optical UV/Vis-sensor, not only in waters with high OC concentrations, but also in the ones with lower C concentrations (< 10 mg C l^{-1}). The highest C-driven disturbance in the sensor NO_x^--N results was found in the bog outlet waters, in which the OC concentrations varied from 13 to 53 mg C L^{-1} for TOC and from 4 to 74 mg C L^{-1} for DOC. The NO_x^--N concentration ranged in the studied waters from 0.04 to 2.5 mg N l^{-1} in the original, mixed and diluted samples from varying surface and spring waters. The studied concentration range is common for Finnish lake and river waters in pristine and agriculturally influenced areas (Räike & Pietiläinen 1999; Eloranta 2004).

Typically, optical sensors are calibrated for the water studied, meaning the measured sensor results (raw data) are corrected with laboratory results. In our study, excluding the bog waters, the 35-mm sensor resulted in 26% and 5-mm sensor in 32% higher NO_x^--N concentrations than the laboratory results (Fig 11.). Similar results were reported also in a study where NO_x^--N values by sensors resulted in a positive bias with increasing DOC concentrations (Drolc and Vrtovšek, 2010).

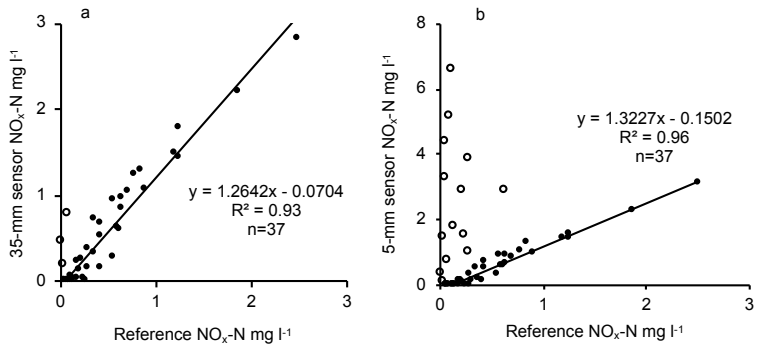

Figure 11. Relation between sensor NO_x^--N and laboratory NO_x^--N results at DOC/ TOC concentrations 0.12–10.0 mg C l^{-1}, a: 35-mm pathlength sensor, b: 5-mm pathlength sensor. The bog outlet waters (empty circles) are excluded from the regression model. The detection limit of the laboratory NO_x^--N analysis is 0.010 mg N l^{-1}. Modified from the study **III**.

We found increasing NO_x^--N error ratios (proportional difference between laboratory and sensor result) under increasing DOC concentrations, which demonstrates the quantity of the disturbance from OC to sensor NO_x^--N results. Steady error ratio indicates that no disturbance from an increasing DOC is found. With both sensors, NO_x^--N error ratio increased with corresponding DOC concentration (0-10.0 mg C l^{-1}) remaining below 2 (0.3-1.6) (Fig. 12). The quality of DOC also affected the NO_x^--N error ratio and in bog water samples, NO_x^--N error ratios were 40-66 (DOC 7-74 mg C l^{-1}) and in bog-river water mixture 1:1 NO_x^--N error ratios were 3.7–15 (DOC 4-40 mg C l^{-1}).

One of the main challenges using optical sensors in NO_x^--N measurements of natural waters is the reduced transmittance of light caused by other substances than NO_x^--N. Indeed, sensor calibration is done to separate the NO_x^--N signal from the interfering signal caused by other substances in the water, referred also as "matrix effects" (Pellerin et al. 2013). NO_x^--N and OC have partially overlapping absorbance regions, as they both absorb UV radiation mainly at wavelengths 200-250 nm (Van den Broeke et al. 2006). Manufacturers offer calibration options which can be done beforehand and these algoritms defined by manufacturer differ depenging on the application, whether the NO_x^--N sensor is intended to be applied in measuring drinking water or wastewater for example. However, it is recommended that local calibration is done to enhance the reliability of the sensor results. One of the most used methods is simple linear regression (SLR) where results obtained

from the reference method, (commonly laboratory results), are plotted against the sensor results, and the obtained linear function is used to correct the sensor results (Huotari & Ketola 2014).

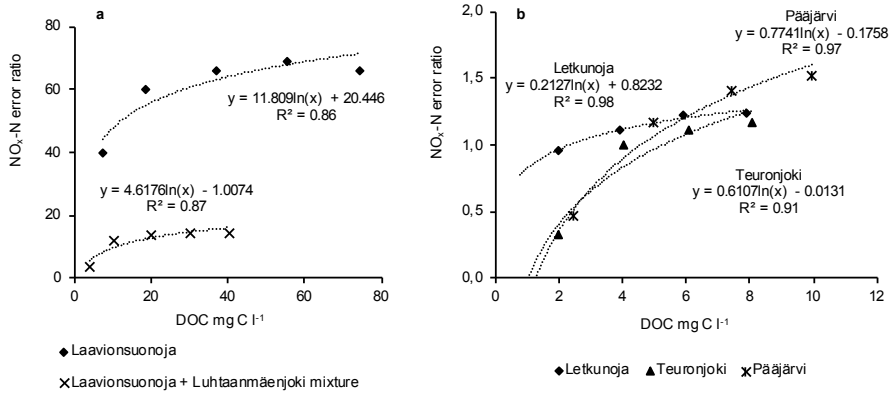

Figure 12. NO_x^--N error ratios with the 5-mm pathlength sensor relative to corresponding DOC concentrations, a: bog outlet waters with high DOC and b: waters with low DOC concentration. Notice the different scales. Modified from the study **III**.

In Brook Koiransuolenoja, the variability between sensor NO_x^--N and laboratory NO_x^--N explained by simple linear function was weak (R^2=0.32). This cannot be explained with spatial variability in the stream channel (Beketov & Liess 2008), as the water samples were taken from the same main flow in the middle of the stream where the optical sensor was installed. We investigated if the interference could be solved by using multiple linear regression (MLR) with DOC added as one of the explanatory variables. We corrected the sensor results with regression function and the sensor data corrected by MLR was more accurate (R^2=0.93, p=0.000) than the sensor data with SLR-correction (Fig. 13). N loading estimates based on traditional sporadic sampling may lead to over- or underestimations, especially in small aquatic systems where temporal and spatial variation in water quality is high (e.g. Linjama et al. 2009; Koskiaho et al. 2010), like found in Koiransuolenoja (Fig. 13; Tikkanen et al. 1985). Using continuous sensors, it is possible to observe the temporal changes in NO_x^--N concentrations leading to more reliable N loading estimates (Koskiaho et al. 2010; Cassidy & Jordan 2011).

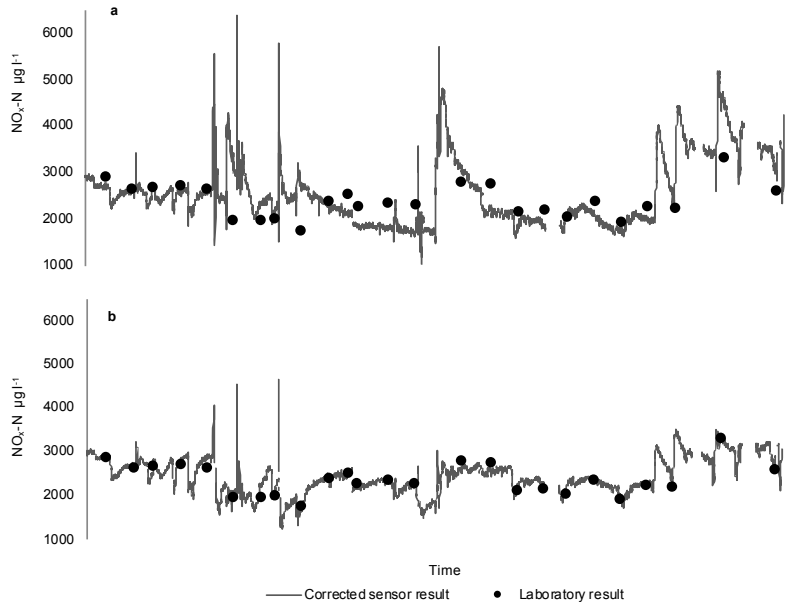

Figure 13. Reference results (laboratory, black circles (n=25) and sensor results (dots) in situ measured in Brook Koiransuolenoja from May to November 2013, a: sensor results corrected with simple linear regression and b: with multiple linear regression. Modified from the study **III**.

In general, the proportion of NO_x^--N of the TN concentration gets higher with increasing proportion of fields in the catchment area (Vuorenmaa et al. 2002; Lepistö et al. 2006) suggesting that NO_x^--N sensors are useful in agricultural catchments. Impacts of agricultural management practices on N and other nutrient loading is challenging to detect with sporadic sampling, but can be assessed more accurately with high-frequency monitoring (Valkama 2018). In addition, continuous *in situ* sensors are able to detect diurnal variation in NO_x^--N concentrations, as well as fluctuations during snowmelt or other runoff peaks (Linjama et al. 2009; Pellerin et al. 2010).

Measuring low NO_x^--N concentrations (0.01-0.43 mg N l^{-1}) with sensors was challenging (**III**), and both sensors indicated only a weak correlation with laboratory results (R^2=0.59 with 35-mm, R^2=0.55 with 5-mm). In NO_x^--N concentrations below 0.28 mg N l^{-1}, both sensors gave zero readings in the spring, lake and river water. This should be taken into account when measuring waters generally with low NO_x^--N, including pristine forest, lake and headwater areas. However, UV/Vis-sensors are important tools in monitoring agricultural or municipal wastewater polluted areas typically showing higher NO_x^--N concentrations.

For NO_x^--N results obtained with optical sensor, significant interference can be caused by substances that have similar absorbance properties to those of OC, such as humic or fulvic acids (Pellerin et al. 2013). Minor proportion of Koiransuolenoja watershed catchment area consists of peatland (5%) and coniferous forests cover most of the catchment area (Arvola et al. 2015), so various organic compounds from both land cover types may have affected in the observed disturbance in the sensor NO_x^--N results. DOC concentrations in Koiransuolenoja (10.6 ±4.5 mg C l^{-1}) are similar to the average concentrations in Finland (Kortelainen 1999). Seasonality and landscape properties have been reported causing variability in OC quantity and quality in boreal lakes (Erlandsson et al. 2012). In many previously low coloured Finnish lakes, the concentrations of humic substances have increased up to 20% during the last decade (FEI 2017) and this "brownification" has been recognized worldwide (Roulet & Moore 2006). One suggested explanation is N deposition, which may enhance the microbial soil decomposition resulting in higher release of organic substances (Findlay 2005). NO_x^--N additions to soils were found to increase the export of DOC, DON and NO_x^--N in a laboratory experiment (Pregitzer et al. 2004). Neverthless, the interference of OC in optical sensor NO_x-N measurements will probably become even more relevant in the future, due to increasingly warm winters and higher precipitation transporting higher OC and NO_x-N loads in the boreal region (Kortelainen et al. 2006).

4 CONCLUSIONS

This dissertation clarifies the possibilities to utilize constructed wetlands in mitigating aquatic N loading in a cold climate. It provides information on the seasonal and diurnal variation of denitrification in shallow agricultural wetland sediments in field conditions with realistic variations of several environmental factors simultaneously. Denitrification rates were governed mainly by temperature followed by oxygen conditions in the sediment and turbidity in the water column. The study (**I**) focused on nitrate-rich, non-vegetated sediment, exposed to diurnal light variation. Potential nitrogen removal estimates based on daytime measurements will most likely result in underestimations. Denitrification was decreased in low temperatures (<+10 ^{o}C) and makes efficient nitrogen removal challenging during high loads in spring and during winter. It should be noted that environmental conditions and governing factors may differ in deeper and vegetated areas of the wetlands. Neverthless, this is the first field study reporting N removal process rates conducted in a Finnish agricultural wetland and provides information of real seasonal denitrification rates under low temperatures in shallow high-nitrate wetlands.

By combining N retention results with actual N removal measurements (**II**), this dissertation estimates the contribution of microbial N removal processes in the total N retention in a constructed wetland polishing treated, high-nitrate municipal wastewater. The constructed wetland retained most of the inorganic nutrient loading and most of the nitrogen retention was based on the microbial gaseous nitrogen production by denitrification. In addition, the inorganic nutrients were transformed into particles through the growth of microalgae. This opens up new possibilities to nutrient recycling and reducing the nutrient loading into receiving waters. The application of constructed wetlands in the Finnish wastewater treatment sector has been mainly neglected. This is one of the first studies to report the possibility to enhance the N retention of the wastewater treatment plants with constructed wetlands in a cold climate. This is important, considering the N removal of municipal wastewaters is highly variable.

Continuous nitrate measurements are important in estimating nitrate loads reliably from small and dynamic agricultural waterways. This study emphasizes the importance of local calibration of optical nitrate sensors due to the potential of organic carbon to interfere with nitrate concentrations measured by sensors **(III**). Generally, the rising concentration of organic carbon in the water increased the error of sensor nitrate results with different surface waters. This error was manifold in bog waters likely arising from the difference in carbon quality. In a small agricultural brook, the local calibration taking account the effect from dissolved organic carbon did overcome the interfering effects arising from organic carbon. This kind of calibration can

enhance the reliability of nitrate load estimates by using a continuous optical nitrate sensor.

Although the rates of nitrogen removal are decreased by low temperature, they are still mostly active during this time. With careful design, constructed wetlands can be used to a greater extent to tackle the excess reactive N load from reaching the vulnerable waterbodies. Continuous nitrate sensors are highly useful in monitoring N loading when properly applied. Future studies in cold climate should focus on seasonal and spatial variation of N removal in high-nitrate wetlands, including the proportional greenhouse gas N_2O production to the total nitrogen removal. Furthermore, the importance of carbon quantity and quality in microbial N removal should be investigated together with high-nitrate and low temperature.

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